ANTI-TUBERCULAR SUBSTANCES PRODUCED BY STREPTOMYCETES

BY

RICHARD PRESSLY MILLER

B.A., Miami University, 1947
M.A., Miami University, 1948

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
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ACKNOWLEDGEMENT

The author wishes to express his sincere appreciation to Dr. H. E. Carter for his invaluable advice and encouragement throughout the course of this work.

The author also wishes to thank Marvin Legator, Mrs. Audrey Bhattacharyya, and Mrs. Bernice Torch for performing the assays during these experiments. The author is indebted to the Upjohn Company for their generous grants and to Dr. David Gottlieb for the culture filtrates of the antibiotics studied.

The author would also like to acknowledge the help of his wife, Betty. Her unfailing cheerfulness and faith made this work possible.

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Introduction

As the search for new antibiotics continues at an ever increasing pace and the list of known ones rapidly lengthens, it becomes increasingly imperative to have available a reliable and convenient method for determining at an early stage whether or not a new isolate produces a known antibiotic. It is the purpose of this thesis to describe the development and application of paper chromatographic methods for the identification of antibiotic substances.

Discussion Of Results

A series of paper chromtaographic systems were established to aid in the screening of new isolates against known antibiotics. Eight ascending solvent systems and one descending system were routinely employed for this purpose. The location of the chromatographed antibiotics was determined by a modification of the technique of Peterson and Reineke (1).

The chromatographic behavior of nine known antibiotics was investigated in the above mentioned solvent systems and these substances were then employed as controls when chromatographing new culture filtrates. The nine antibiotics thus used were actinomycin, chloromycetin, clavacin, aureomycin, terramycin, neomycin, streptomycin, streptothricin, and streptolin.

Nine culture filtrates containing antibiotics which were not isolated were screened in this manner. In the case of antibiotic 61-57 all possibilities were eliminated with the exception of chloromycetin and actinomycin. Two others, antibiotics 66-36 and 41-31 were shown to be terramycin on the basis of their chromatographic behavior and supporting physical and microbiological data. Of five new isolates from soil sample 67, one,

antibiotic 67-41, was shown to be none of the nine known antibiotics listed above. A second, antibiotic 67-27, appeared to be similar to the streptomycin, streptothricin, streptolin group of antibiotics in its chromatographic behavior, but did not appear to be identical with any of that group. The other three did not exhibit any significant difference in their chromatographic behavior from that shown by streptomycin, streptothricin and streptolin. The chromatographic behavior of antibiotic 70-2 indicated that it was probably not identical with any of these three known antibiotics.

Five anti-tubercular substances produced by streptomycetes were isolated. One of these, antibiotic 46-37, was isolated by percolating the filtered brew through an Amberlite IRC-50 column buffered at pH 7. The antibiotic was eluted from the column with 0.1 N hydrochloric acid, the eluate neutralized with sodium hydroxide and then lyophilized. The solid thus obtained was repeatedly extracted with methanol and a partial purification achieved by passage of this methanol solution over an alumina column. Paper chromatographic studies indicated that this antibiotic contained two active components which are probably identical with two of the members of the streptolin complex. The LD_{50} of the crude material for mice was found to be approximately 7.5 mg./kg.

Antibiotic 55-90 was isolated by extracting the filtered brew with *n*-butanol. The butanol extracts were taken to dryness and the crude solid thus obtained was partially purified by passage over a Celite column using *n*-butanol:water as the solvent system. This antibiotic was identified as terramycin on the basis of results obtained from chromatographic studies, counter-current distribution studies, color tests, ultraviolet spectra, antibacterial spectrum studies and other microbiological and chemical data.

Three other antibiotics, 65-24, 65-53 and 65-104, were shown to be different from each other and from any of the nine known antibiotics listed above on the basis of their chromatographic behavior. Antibiotic 65-24 was isolated by extracting

the filtered brew with ethyl acetate. Upon concentrating the extract to dryness, a yellow solid was obtained which had good antibiotic activity. A partial purification was achieved by one reprecipitation from benzene with *n*-hexane. The yellow powder thus obtained had a melting point range of 185-195°.

Antibiotics 65-53 and 65-104 were both isolated by extracting the filtered brew with *n*-butanol. Upon concentrating the extracts to dryness, a tan solid was obtained in both cases which had fair antibiotic activity. It was found that aqueous solutions of antibiotic 65-104 could be deionized and decolorized by ion-exchange resins.

A sixth anti-tubercular substance, antibiotic 51-38, was not isolated. However it was found to be adsorbed by a number of substances, to be non-dialyzable and to be precipitated by lead acetate. It was also found that antibiotic 51-38 could be regenerated from its lead salt with no loss in activity by means of Amberlite IR-120.

Summary

1. A series of paper chromatograhpic systems designed to aid in the early identification of antibiotics has been described. The usefulness of this system in an antibiotic screening program has been discussed and illustrated.

2. The isolation of five anti-tubercular substances produced by streptomycetes has been described and some of the properties of a sixth reported.

3. One of these, antibiotic 46-37, was shown to contain two active components which are probably identical with two of the members of the streptolin complex.

4. A second, antibiotic 55-90, was shown to be terramycin on the basis of a large amount of physical, chemical and microbiological data.

5. Three others, antibiotics 65-24, 65-53 and 65-104 were shown to be different from each other and from any of the well

known antibiotics.

6. The sixth, antibiotic 51-38, was not isolated but was found to be non-dialyzable and to be precipitated by lead acetate.

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VITA

Richard Pressly Miller was born on February 18, 1922 in Troy, Ohio. After graduating from Van Cleve High School in 1940, he entered Miami University, Oxford, Ohio. He interrupted his undergraduate training at that institution in 1942 in order to enlist in the United States Army Air Corps. After serving in the American and Southwestern Pacific Theaters he was discharged from the Air Corps in 1945. In 1946 he returned to Miami University and received the degree of Bachelor of Arts, with honors, from that institution in August, 1947. He began his graduate work there at that time and received the degree of Master of Arts in July, 1948. He entered the Graduate College of the University of Illinois in September of the same year. He held a half-time teaching assistantship for two semesters, and an Upjohn Research Fellowship from September, 1949 to July, 1951.

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